Two-site Evaluation of a Rapid and Simple *CFTR* PCR/CE Assay and Software Targeting Mutations Across Diverse Ethnic Groups

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Summary

- Cystic Fibrosis (CF) is caused by mutations in both copies of the *CFTR* gene, and affects ~1 in 3000-4000 US births; more than 2000 *CFTR* variants have been identified, with variable frequency within different population groups.
- We developed a streamlined, two-tube PCR/CE prototype assay which detects 67 variants commonly found in the diverse US population.
- Evaluation of AmplideX® PCR/CE *CFTR** prototype assay produced consistent results for 73 residual clinical samples and eight controls across two laboratories, using a faster and simpler workflow than many other assays.

Introduction

Cystic Fibrosis is a progressive, hereditary disease characterized by the accumulation of viscous mucus in multiple organs and caused by mutations in the *CFTR* gene. Although over 2000 genetic mutations have been reported, only ~300 of these are pathogenic, and their prevalence is dependent on ethnicity. Per recent ACMG guidelines, a 23-variant panel is minimally recommended for targeted testing. Here, we describe a targeted assay that addresses >92% mutant prevalence represented in different databases and across diverse populations (CFTR2 database, gnomAD, Beauchamp et al. 2019) and detects 67 pathogenic variants, including the ACMG-23. We report a two-site evaluation of a prototype of this PCR/CE-based assay using independently genotyped, residual clinical samples.

Methods

Seventy-three previously genotyped residual clinical samples and eight controls (multi-variant mixes of cell lines and synthetic gBlock templates) were assayed by two laboratories. Samples were amplified in a two-tube assay using AmplideX® PCR/CE *CFTR* Prototype reagents. Allele-specific PCR generates mobility- and dye-tagged amplicons that are resolved by variant type. PCR products were resolved by capillary electrophoresis (CE) on the Applied Biosystems™ 3500xL; one site also analyzed a subset of samples on 3130xl, 3730xl, and SeqStudio™ Genetic Analyzers. Genotypes were determined by prototype modules in GeneMapper 5.0, and compared using automated peak calling and classification software at one site. Each sample batch was processed within five hours from PCR setup through CE and peak calling.

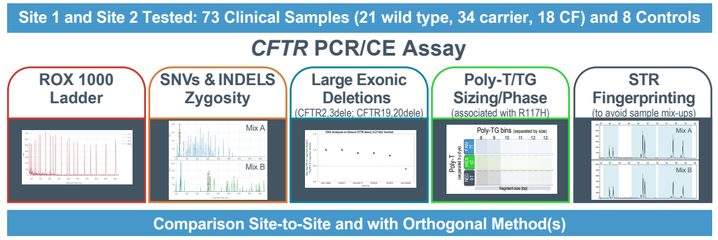

Figure 1. Study Overview and Elements of the *CFTR* PCR/CE Assay. Samples were tested at two sites and results were compared site-to-site, and with orthogonal methods (xTAG® Cystic Fibrosis (*CFTR*) 60 Kit v2, and Sanger sequencing where appropriate). The *CFTR* PCR/CE prototype assay reports variants/zygosity status along with Poly-T/TG sizing from the unified workflow, with ROX ladder sizing and STR fingerprinting included in each tube.

**This product is under development. Future availability and performance to be determined.*

Presented at AMP 2020

Results

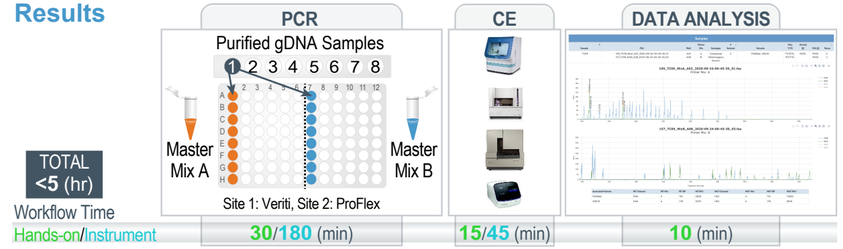

Figure 2. *CFTR* Assay Workflow and Turnaround Time. The assay system combines kitted reagents with peak calling and classification software, resulting in a streamlined workflow that can be performed in <5 hours from DNA to genotype with <1 hour of hands on time.

Number of Variants

Unique to xTAG® *CFTR* 60 Kit v2 (17)		Variants in Both Kits (47)				Unique to *CFTR* PCR/CE Kit (20)	
S549R(1)	K710X	F508del	3120+1G>A	M1101K	1898+5G>T(2)	R117C	1548delG
2307insA	S1196X	G542X	R1162X	Y1092X	CFTRdele2,3	3272-26A>G	P5L
F508C	2055del9>A	G551D	G85E	3905insT	L206W	P67L	R1070W
I506V	935delA	N1303K	3659delC	S549N	D1152H	1811+1.6kbA>G	V456A
I507V	3199del6	R117H	R347H	R347P	E60X	R352Q	R1070Q
S1255X	406-1G>A	W1282X	A455E	1078delT	R1066C	F312del	G970D
2143delT	G330X	621+1G>T	R334W	V520F	R1158X	IVS8 PolyTG	2657+3insA
G178R	3791delC	R553X	1898+1G>A	Y122X	1677delTA	2184insA	I618T
W1089X		1717-1G>A	R560T	IVS8 PolyT	Q890X	1154insTC	CFTRdele20
		3849+10kbC>T	394delTT	2183AA>G	Q493X	S945L	G622D
		2789+5G>A	711+1G>T	3876delA	R75X		
0.4% per Beauchamp		I507del	2184delA	A559T		**7.0% per Beauchamp**	
		86.1% per Beauchamp et al. (2019)					

Figure 3. Variant coverage. The *CFTR* PCR/CE assay covers 67 variants representing 93% of variant alleles in the ethnically diverse US population (Beauchamp et al., 2019). Of those, 47 variants are common with the orthogonal *CFTR* assay used in this study. The clinical sample set included 21 different *CFTR* variants (marked in red) that were common to both assays. Two samples that were part of the original sample set (75 samples collected over 8 years) were excluded due to variants not detected by both kits (marked in blue).

Table 1. Comparison Site-to-Site and with an Orthogonal Method, using 3500xL genetic analyzer and GeneMapper analysis. The prototype *CFTR* Panel demonstrated 100% agreement across 73 Clinical samples between the sites and when compared to an orthogonal panel. A subset of samples was also tested on three additional CE platforms at one site with matching results.

Sample Agreement with Orthogonal Assay			xTAG® Cystic Fibrosis (*CFTR*) 60 Kit v2			Overall Sample Agreement
			Homozygous or Compound Heterozygous		Heterozygous	
			WT/WT	MUT/MUT	MUT/WT	
Prototype *CFTR* PCR/CE Assay	Homozygous or Compound Het	WT/WT	**21**	0	0	**21/21(100%)**
		MUT/MUT	0	**18**	0	**18/18 (100%)**
	Heterozygous	MUT/WT	0	0	**34**	**34/34 (100%)**

Figure 4. Example Electropherograms. In-development *CFTR* analysis module, incorporated into AmplideX® PCR/CE Reporter software, was used for automated peak calling and sample classification to analyze fsa files generated at two sites. Results were compared to reference data. Matching results were obtained for 146/146 clinical sample files (100% agreement).

Conclusions

- We developed and evaluated an AmplideX PCR/CE *CFTR* prototype assay that covers 93% variants from the diverse US population, including all targeted mutations recommended by ACMG/ACOG guidelines.
- The prototype AmplideX PCR/CE *CFTR* assay has a rapid workflow, requiring only five hours from sample-to-answer with the flexibility to support high-throughput screening or lower-volume diagnostic applications.
- We observed 100% genotype agreement to reference results from 73 residual blood samples at two labs, including SNPs, INDELs, CNVs, and the IVS8 poly-T/TG modifier.
- Prototype automated software results were 100% concordant with reference results and incorporated multiple QC checks, matched-tube assignment, and automated peak calling/classification.

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